

What Is Claimed Is:

1. A method of purifying recombinant human erythropoietin from cell culture supernatants comprising by a combination of the following steps:

5 (a) differential saline precipitation; ✓
(b) hydrophobic interaction chromatography; ✓
(c) concentration and diafiltration; ✓
(d) anionic exchange chromatography; ✓
(e) cationic exchange chromatography;
(f) concentration and diafiltration; ✓
(g) molecular exclusion chromatography. ✓

10 2. The method of Claim 1, wherein steps a) through g) are performed in the following order: (a), (b), (c), (d), (e), (f) and (g).

15 3. The method of Claim 1, wherein steps a) through g) are performed in the following order: (a), (c), (d), (e), (b), (f) and (g).

20 4. The method of Claim 1, wherein step a) comprises adding ammonium sulfate to said culture supernatant, followed by centrifugation.

25 5. The method of Claim 1, wherein step (b) comprises using a hydrophobic interaction matrix.

6. The method of Claim 5, wherein said hydrophobic interaction matrix employed is Phenyl Sepharose 6 Fast Flow.

7. The method of Claim 1, wherein step (d) comprises using an anionic exchange matrix.

30 8. The method of Claim 7, wherein said anionic exchange matrix is Q-Sepharose Fast Flow.

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9. The method of Claim 1, wherein step (e) comprises using a cationic exchange matrix.

10. The method of Claim 9, wherein said cationic exchange matrix is SP-
5 Sepharose Fast Flow.

11. The method of Claim 1, wherein step (g) comprises using a molecular exclusion matrix.

12. The method of Claim 11, wherein said molecular exclusion matrix employed is Sephadryl S-200 HP.

13. A substantially pure erythropoietin, produced according to the method of
Claim 1.

14. The erythropoietin according to Claim 13, wherein said EPO has a purity exceeding 99% as determined by a polyacrilamide gel electrophoresis analysis (SDS-PAGE) and reverse phase and molecular exclusion liquid chromatography.

20 15. The erythropoietin according to Claim 13, wherein said EPO is characterized by a series of isoforms of isoelectric point values between 3.0 and 4.5.

25 16. The erythropoietin according to Claim 13, wherein said EPO shows homology to the amino acid sequence of SEQ ID NO:1.